Hot-Water Immersion Quarantine Treatment Against Mediterranean Fruit Fly and Oriental Fruit Fly (Diptera: Tephritidae) Eggs and Larvae in Litchi and Longan Fruit Exported from Hawaii

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ABSTRACT Immersion of litchi fruit in 49°C water for 20 min followed by hydrocooling in ambient (24 ± 4°C) temperature water for 20 min was tested as a quarantine treatment against potential infestations of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann); and oriental fruit fly, *Bactrocera dorsalis* Hendel, eggs or larvae in Hawaiian litchi, *Litchi chinensis* Sonnerat. The 49°C hot-water immersion of litchi provided probit 9 (99.9968% mortality with >95% confidence) quarantine security against eggs and first instars. There were no survivors from 15,000 each feeding and nonfeeding Mediterranean fruit fly or oriental fruit fly third instars immersed in a computer-controlled water bath that simulated the litchi seed-surface temperature profile during the 49°C hot-water immersion treatment. Litchi served as the model for longan, *Dimocarpus longan* Lour., a closely related fruit that is smaller and also has commercial potential for Hawaii. Modified fruit infestation and holding techniques used to obtain adequate estimated treated populations from poor host fruit, such as litchi and longan, are described. Data from these experiments were used to obtain approval of a hot-water immersion quarantine treatment against fruit flies for litchi and longan exported from Hawaii to the U.S. mainland.

KEY WORDS *Litchi chinensis*, fruit flies, quarantine pests, postharvest treatment

Litchi (syn. lychee, leechee), *Litchi chinensis* Sonnerat, fruit is a recorded host of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and oriental fruit fly, *Bactrocera dorsalis* Hendel, although natural infestations produce little or no survival to the adult stage (Liquido et al. 1991, Follett and McQuate 2001). Because Mediterranean fruit fly and oriental fruit fly occur in Hawaii, litchi fruit cannot be exported from Hawaii to the U.S. mainland without quarantine treatment. Litchi is not recorded as a host for melon fly, *Bactrocera cucurbitae* (Coquillett), or the so-called "Malaysian or solanaceous fruit fly," *Bactrocera latifrons* (Hendel), which also occur in Hawaii.

Before 1984, the only quarantine treatment to control potential fruit fly infestations in litchi was fumigation with ethylene dibromide. Subsequently, ethylene dibromide was banned as a postharvest fumigant (Ruckelshaus 1984). Presently, litchi can be exported from Hawaii to U.S. mainland markets after a 150-Gy irradiation quarantine treatment to kill or sterilize any potential fruit fly infestations (Follett and Armstrong 2004, Federal Register 2006). Hot-water immersion quarantine treatments have been developed for banana (*Musca* spp.) and papaya, *Carica papaya* L., against Mediterranean fruit fly, melon fly, and oriental fruit fly; for carambola, *Averrhoa carambola* L., grapefruit, *Citrus paradisi* Macf., and guava, *Psidium guajava* L., against Caribbean fruit fly, *Anastrepha suspensa* (Loew); and for mango, *Mangifera indica* L., against *Anastrepha distincta* Greene, *Anastrepha serpentina* (Wiedemann), Caribbean fruit fly, Mediterranean fruit fly, *Anastrepha ludens* (Loew), *Anastrepha fraterculus* (Wiedemann), and *Anastrepha obliqua* (Macquart) (Armstrong 1994). The hot-water immersion time and temperature combinations required to provide quarantine security varies with fruit fly species and host fruit, and they are in the range of 46–50°C. The temperature used in any particular treatment was chosen for least deleterious effects to fruit quality as well as for quarantine security (Armstrong 1994). For example, a hot-water immersion quarantine treatment for the 'Brazilian' banana consists of immersing fruit in 50°C (122°F) water for 15 min (Armstrong 1982), whereas a hot-water immersion quarantine treatment for mangoes consist of immersing fruit in 46°C water for 90 min (Nascimento et al. 1992).

Hot-water immersion was selected as the candidate heat treatment for litchi over vapor heat because the heat transfer coefficient of liquid water is greater than that of the vapor phase (Armstrong and Couey 1989); therefore, fruit heat faster in a hot-water immersion treatment than in a vapor heat treatment. Exported litchi fruit treated with hot water can be certified as organic, whereas those treated with irradiation do not

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qualify for this certification (National Organic Program 2007). A hot-water immersion temperature of 49°C was chosen originally for Hawaii-grown litchi because of potential convenience. Hot-water immersion treatment tanks with 49°C water were already in use in the Hawaii papaya industry as a postharvest decay control method (Akamine and Arisumi 1953). Preliminary quality checks were done in cooperation with the Hawaii litchi industry. Fruit provided by the industry were immersed in 49°C water for 20 min followed by 20-min hydrocooling in ambient (24 ± 4°C) temperature water, and then they were stored at 10°C for 2 d to simulate air freight conditions between Hawaii and the U.S. mainland. The industry reported that the litchi tolerated the treatment with little or no adverse effects to fruit quality.

Longan (syn. longan, longyen, "dragon's eye"), *Dimocarpus longan* Lour., is closely allied with litchi in the family Sapindaceae (Watson 1984, Morton 1987). Both fruits share the same basic morphology of a thin pericarp and edible aril surrounding a single seed; however, the litchi pericarp is rough and red, whereas the longan pericarp is brown, leathery, and smooth. Longan fruit is typically smaller than litchi. Like litchi, longan is attacked by Mediterranean fruit fly and oriental fruit fly and is a poor host to both (Follett and McQuate 2001). Longan currently is being exported from Hawaii to the U.S. mainland by using irradiation and would benefit from an organic alternative quarantine treatment.

We report here studies to determine whether immersion of litchi in 49°C water for 20 min followed by 20-min hydrocooling in ambient (24 ± 4°C) temperature water would provide adequate quarantine security against Mediterranean fruit fly or oriental fruit fly eggs and larvae. Melon fly was included in all tests for the purpose of comparing its relative heat tolerance. Heating profiles for longan and litchi were compared to determine whether the hot-water immersion treatment for control of fruit flies in litchi also would be effective in longan.

Materials and Methods

Test Fruit. Litchi used in all hot-water immersion treatment tests were collected from orchards located in Hilo, Puna, Ka'u, or Kailua-Kona districts, Hawai'i county; Hanalei or Kawaihau districts, Kaua'i county; or Wahiawa district, Honolulu county; and they were brought to the USDA–ARS laboratory in, Hilo, HI. Fruit were used either immediately or refrigerated (10°C) until use. Refrigerated fruit were warmed to 21 ± 3°C before they were used in tests. Only undamaged, firm fruit were used; overripe, damaged or diseased fruit were discarded. Cultivars of litchi used in hot-water immersion treatment tests included 'Brewster', 'Groff', 'Kaimana', and 'Tai So', and individual fruit weights averaged 27.3 ± 4.4 (mean + SD), 12.2 ± 2.8, 24.1 ± 4.3, and 20.2 ± 2.8 g, respectively.

Test Insects. Fruit fly species used in fruit infestations were Mediterranean fruit fly, melon fly, or oriental fruit fly. Although litchi is not recognized as a melon fly host, this fruit fly species was included in our hot-water immersion treatment tests to collect ancillary data. Fruit fly eggs used to infest litchi were collected from laboratory-reared adults (Vargas 1989) in devices similar to those described by Hart and Miyabara (1968). Eggs not used to infest fruit were held on moist blotter paper until hatch to obtain first instars. Eggs and/or first instars not used to infest fruit were placed on larval diet (Vargas 1989) to obtain third instars. Feeding and nonfeeding third instars used in hot-water immersion tests were collected by flotation in water as described by Jang (1991).

Fruit Infestation Method. Because litchi is a poor fruit fly host and natural infestations produce little or no viable survivors, litchi fruit were infested by injecting ≈500 Mediterranean fruit fly, melon fly, or oriental fruit fly eggs or first instars into the fruit flesh ≈0.5 cm below the fruit surface of each fruit by using a 1-ml syringe with a 16-gauge needle (Kamburov 1972, Armstrong et al. 1995b). After treatment, the eggs or first instars were irrigated out of the fruit flesh with a fine-jet wash bottle containing tap water onto moist larval diet (Armstrong et al. 1995b).

Hot-Water Immersion Treatment of Litchi Fruit Infested with Eggs and First Instars. Approximately one quarter to one half of the fruit infested with eggs or first instars were held as controls (the amount of fruit held for controls varied with the amount of fruit that was available for tests). The remaining infested fruit were placed in 2.5-cm^2-mesh welded wire baskets (15.2 by 38.1 by 15.2 cm, width by length by height) divided into three equal compartments. For each replicate, each basket compartment contained an average of 1,486.3 ± 32.3 g of Brewster, 1,357.7 ± 48.1 g of Groff, 1,537.8 ± 34.5 g of Kaimana, or 1,326.6 ± 34.7 g of Tai So fruit. The baskets of infested fruit were immersed for 20 min in a 70-liter circulating bath heated by two electric heaters (immersion circulator model 73, PolyScience, Niles, IL) to a constant 49 ± 0.2°C (temperature monitoring equipment and methods are described below). The bath temperature was verified before and after each test by using a mercury thermometer with 0.1°C gradations. After the 20-min immersion in 49°C water, the treated fruit were immediately hydrocooled in circulated ambient (24 ± 4°C) temperature water for 20 min.

Water and fruit seed-surface temperatures were monitored using 32-gauge copper-constantin thermocouples attached to a datalogger (Polycorder model 516C, Omnidata International, Logan, UT). Thermocouple tips were inserted into uninfested fruit through a small hole in the fruit skin until it abutted the seed. The area of thermocouple insertion on the fruit skin was sealed with silicon sealant (Dow Corning, Midland, MI), and the thermocouples were held in place with masking tape. The fruit with thermocouples were interspersed in the baskets holding infested fruit.

When the treatment was completed, the control fruit and the hydrocooled treated fruit were cut open using a scalpel to expose the fruit flesh where the eggs or first instars were injected. The eggs or first instars were irrigated out of the fruit flesh onto moist larval

diet. The control and treated fruit from which eggs or first instars were irrigated were placed on trays of dry larval diet in holding cabinets for 2–3 wk (Armstrong et al. 1984) to permit the development of any surviving eggs or larvae within the fruit that might have been overlooked during the irrigation procedure. All remaining fruit debris and larval diet was thoroughly searched for surviving larvae and pupae at the end of the holding period.

Seed-Surface Temperature Profile and Hot-Water Immersion Treatment of Third Instars. The 49°C hot-water immersion treatment was tested against third instars in vitro by using computer-controlled variable-temperature water baths described by Shellie et al. (1993) that simulated the litchi seed-surface temperature profile recorded during the hot-water immersion treatment and subsequent hydrocooling. In general, the test conditions used for quarantine treatment research should mimic proposed commercial treatment conditions as closely as possible. However, in this case, obtaining litchi fruit infested either naturally or artificially with third instars for in situ testing of the hot-water immersion treatment was impractical. Natural infestation by exposing litchi fruit in cages containing large numbers of gravid female fruit flies yielded unacceptably low infestation rates (J.W.A., unpublished data). Furthermore, fruit containing third instars from natural infestations cannot be used in hot-water immersion treatment tests because fruit deterioration is too advanced and the fruit disintegrate during treatment. Attempts at artificial infestation with third instars were unsuccessful, because a relatively low percentage of insects developed into adults under these conditions (J.W.A., unpublished data). The small fruit size and watery flesh found in litchi prevented artificial infestation with third instars, such as the method described by Armstrong et al. (1995a, 1995b) for carambola and papaya. Removal of enough fruit flesh to infest litchi with third instars irreparably damaged fruit skin integrity and radically altered the fruit interior and heat transfer characteristics.

The water bath heating and cooling temperature profile was obtained from the mean of 29 temperature profiles recorded from litchi seed surfaces, including five, six, nine, and nine temperature profiles recorded from Brewster, Groff, Kaimana, and Tai So seed surfaces, respectively, during 49°C hot-water immersion treatment and subsequent hydrocooling in 24 ± 4°C water. Water temperatures were monitored and controlled with the Water Troll Controlled-Temperature Water Baths computer program (Gaffney 1990). "Set" temperatures, which fall between 60-s readings, were determined by interpolation by using a parabolic function (Shellie et al. 1993).

To determine the amount of time required to obtain 100% mortality of third instars during only the heating phase of the 49°C hot-water immersion treatment, 25 exposed (naked) Mediterranean fruit fly, melon fly, or oriental fruit fly feeding or nonfeeding third instars were placed in black muslin cloth containers constructed from open plastic syringe tubes (30 ml) (Jang 1991). Four containers of larvae were placed in the computer-controlled variable-temperature water bath at the beginning (0 min) of the 49°C hot-water immersion treatment profile. One container each of larvae was removed at 5-min intervals for the duration of the treatment (i.e., at 5, 10, 15, and 20 min, with 20 min the end of the treatment), and it was immediately immersed in ambient (24 ± 4°C) temperature water for 1 min. Each test used 25 insects per treatment time for each fruit fly species and feeding stage, and each test was replicated 10 times for a total of 250 insects per treatment. For each test, 100 feeding or nonfeeding third instars were held in ambient temperature water for 21 min as controls. Control and treated feeding third instars were placed on larval rearing diet as described by Armstrong et al. (1995b), and they were held until pupation was completed. Control and treated nonfeeding third instars were placed directly on sand to pupate. The treatment test was replicated ten times each for feeding and nonfeeding third instars for each fruit fly species.

To determine the efficacy of the overall hot-water immersion treatment, including the hydrocooling phase, 100 feeding or nonfeeding Mediterranean fruit fly, melon fly, or oriental fruit fly third instars were placed in each of three black muslin cloth containers. The containers with larvae were placed in the computer-controlled variable-temperature water bath at the beginning of the 20-min heating phase and removed at the end of the 20-min hydrocooling phase of the 49°C hot-water immersion treatment profile. One hundred feeding or nonfeeding third instars were held in ambient (24 ± 4°C) temperature water for 40 min as controls. Control and treated third instars were handled as described for the sublethal heat mortality tests. The 49°C hot-water immersion treatment tests were replicated 50 times (i.e., a treated population of 15,000) each for feeding and nonfeeding third instars of each fruit fly species.

Treatment Efficacy. The criterion for effectiveness of the treatment was based on the number of normal pupae that developed from the treated eggs and larvae (Baker 1939, Hill et al. 1988, Sharp and Picho-Martinez 1990). For eggs and first instars injected into fruit, the effective number of treated flies was estimated by dividing the number of fruit flies recovered from the control fruit by the weight of the control fruit and then multiplying the quotient by the weight of the treated fruit (Balock et al. 1966).

Armstrong et al. (1989) determined that Mediterranean fruit fly, melon fly, and oriental fruit fly third instars were less tolerant to heat treatment than were eggs or first instars. Therefore, large-scale validation tests were conducted with eggs and first instars only. The probit 9 standard (99.9968% mortality) was used to evaluate the effectiveness of the hot-water immersion treatment (Baker 1939). To achieve probit 9 efficacy at the 95% confidence level, a minimum of 93,613 insects must be tested with no survivors (Couey and Chew 1986). Quantitative methods have been developed to calculate the number of test insects and confidence limits (CL) for other levels of precision and treatment efficacy, with and without survivors

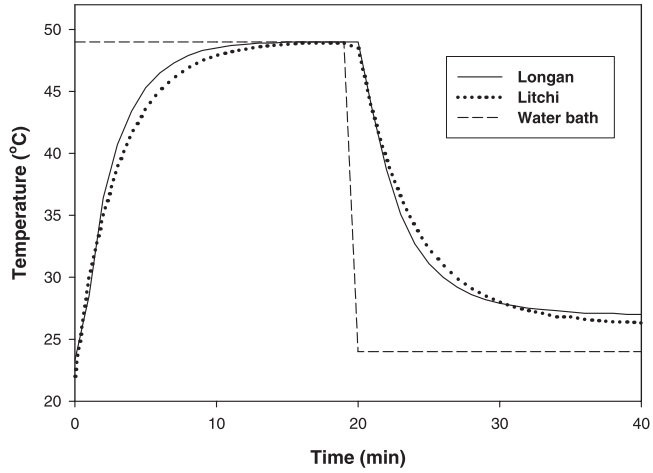

Fig. 1. Temperature profile for typical litchi and longan fruit subjected to hot-water immersion at 49°C for 20 min followed by hydrocooling in ambient temperature water for 20 min. Temperatures were measured at the seed surface inside fruit.

(Couey and Chew 1986, Follett and McQuate 2001). Couey and Chew (1986) provide an equation to estimate the confidence levels for efficacy when only a few insects survive on a treated host,

$$\sum_{x=0}^{x=s} e^{-m} m^x / x! = 1 - C$$

where m is npu, n is the number of insects tested, pu is the maximum allowable infestation proportion (e.g., 0.000032 for 99.9968% mortality), s is the number of survivors, and C is the confidence level. This equation uses the Poisson distribution law and assumes large n and small p_u (Couey and Chew 1986). For eggs and first instars, CQT_STATS software (Liquido et al. 1996) was used to calculate probit 9 confidence levels for various sample sizes with and without survivors.

Probit analysis was done on untransformed data from studies with feeding and nonfeeding third instars (LeOra Software 1987, Robertson and Preisler 1992). For each species and feeding stage, data used in the probit model included any hot-water immersion dose causing mortality between 0 and 100%, and the lowest dose causing 100% mortality. Significant differences between feeding and nonfeeding stage third instars for each species were determined by nonoverlap of 95% CL.

Results

Litchi Fruit Infested with Eggs and First Instars. Fig. 1 shows the average seed-surface temperature profile developed from litchi and longan fruit immersed in 49°C water for 20 min followed by hydrocooling in ambient (24 ± 4°C) temperature water for 20 min. The mean litchi fruit weight (20.8 g) was almost twice the mean longan fruit weight (11.5 g). Litchi heated to 49°C and cooled more slowly than longan due to its larger size.

Table 1 shows the survival of Mediterranean fruit

Table 1. Survival of Mediterranean fruit fly, melon fly, or oriental fruit fly eggs or first instars in litchi fruit immersed in 49°C water for 20 min followed by hydrocooling in 24 ± 4°C water for 20 min

Species and life stages	No. replications	Fruit wt (kg)		No. survivors		Estimated treated pop[a]	Confidence level for probit 9[b]
		Control	Treated	Control	Treated		
Mediterranean fruit fly							
Egg	13	3.43	3.53	139,092	1	146,404	0.95
First instar	24	11.46	60.74	33,225	3[c]	213,898	0.91
Total	37	14.89	64.27	172,317	4	360,302	0.99
Melon fly							
Egg	10	2.47	2.39	131,995	0	127,161	0.98
First instar	7	2.53	23.12	37,277	1	361,289	1.0
Total	17	5.00	25.51	169,272	1	488,450	1.0
Oriental fruit fly							
Egg	10	2.66	2.70	164,856	1	168,664	0.97
First instar	16	9.58	36.41	63,706	5[c]	356,392	0.97
Total	26	12.24	39.11	228,562	6	525,056	0.99

[a] The estimated treated population for each replication was calculated and the values summed.
[b] The confidence level that the treatment caused 99.9968% mortality.
[c] Survivors were single individuals in different test replications.

Table 2. Percentage of survival of Mediterranean fruit fly, melon fly, and oriental fruit fly feeding and nonfeeding third instars immersed for 5, 10, 15, or 20 min in a computer-controlled water bath heated to simulate the seed-surface temperature profile of litchi fruit immersed in 49°C water for 20 min

Fruit fly species	Third instar stage[a]	Total % survival: Min in water bath from beginning of temp profile				
		0[b]	5	10	15	20
Mediterranean fruit fly	Feeding	88.0[c]	74.8	59.6	0.4	0.0
	Nonfeeding	83.7	70.0	0.4	0.0	0.0
Melon fly	Feeding	94.9	95.6	76.4	3.2	0.0
	Nonfeeding	93.4	91.2	77.6	0.0	0.0
Oriental fruit fly	Feeding	96.9	96.0	97.2	9.2	0.0
	Nonfeeding	88.9	76.8	58.4	0.4	0.0

[a] Feeding larvae were removed directly from rearing diet; nonfeeding larvae were collected after they left the rearing diet before beginning pupation.
[b] Control larvae were immersed in ambient (21 ± 3°C) temperature water for 20 min; treated larvae were cooled in ambient temperature water for 1 min immediately after treatment.
[c] Percentage of survival of control and treated larvae based on 10 replications with 25 larvae per exposure time to the temperature profile per test replication.

Table 4. Mortality of Mediterranean fruit fly, melon fly, and oriental fruit fly feeding or nonfeeding third instars immersed in a computer-controlled water bath heated to simulate the seed-surface temperature profile of litchi fruit immersed in 49°C water for 20 min[a]

Fruit fly species	Treatment	No. survivors from 15,000 larvae[b]	
		Feeding[c]	Nonfeeding[c]
Mediterranean fruit fly	Control[d]	12,222	14,387
	Treated	0	0
Melon fly	Control	13,420	14,169
	Treated	0	0
Oriental fruit fly	Control	13,923	14,225
	Treated	0	0

[a] Treatment included 20-min heating phase followed by 20-min hydrocooling phase.
[b] Total of 50 replications with 300 control and 300 treated larvae in each replication.
[c] Feeding larvae were removed directly from rearing diet; nonfeeding larvae were collected after they left the rearing diet before beginning pupation.
[d] Control larvae were immersed in ambient (21 ± 3°C) temperature water for 40 min.

fly, melon fly, or oriental fruit fly eggs and first instars in litchi fruit immersed in 49°C water for 20 min followed by hydrocooling in 24 ± 4°C water for 20 min. One Mediterranean fruit fly egg and three first instars survived from estimated treated populations of 146,404 and 213,898, respectively. No melon fly eggs and one first instar survived from estimated treated populations of 127,161 and 361,289, respectively. One oriental fruit fly egg and five first instars survived from estimated treated populations of 168,664 and 356,392, respectively (Table 1). All fruit fly survival from the treated fruit consisted of single individuals surviving in different test replications. Treatment efficacy met or exceeded probit 9 efficacy at the 95% confidence level for the eggs of all three species, and for the first instars of melon fly and oriental fruit fly (Table 1). After combining eggs and first instars, the probit nine quarantine security standard was met at the >99% confidence level for each species (Table 1).

Simulated In-fruit Treatment of Third Instars. Table 2 shows percentage of survival in tests with Mediterranean fruit fly, melon fly, or oriental fruit fly feeding or nonfeeding third instars immersed for 5, 10, 15, or 20 min in a computer-controlled water bath heated to simulate the heating phase of the seed-surface temperature profile shown in Fig. 1 for litchi. The rate of pupation of feeding or nonfeeding third instars was greatly reduced in the 15-min treatment, and no third instars successfully pupated in the 20-min treatment (Table 2). LT_{50} values for Mediterranean fruit fly and oriental fruit fly were significantly higher in feeding third instars than nonfeeding third instars, suggesting this stage is more tolerant of heat (Table 3). The predicted immersion time at 49°C to achieve 99.9968% mortality of third instars ranged from 8.4 min for nonfeeding stage Mediterranean fruit flies to 16.9 min for feeding stage oriental fruit flies (Table 3).

Table 4 shows the results of 15,000 each Mediterranean fruit fly, melon fly, or oriental fruit fly feeding or nonfeeding third instars immersed in a computer-controlled water bath heated to simulate both the heating and hydrocooling phases of the seed-surface temperature profile shown in Fig. 1. There were no survivors in this treatment.

Table 3. Linear regression of percentage of mortality against immersion time for Mediterranean fruit fly, oriental fruit fly, and melon fly third instars immersed in 49°C water

Fruit fly species	Third instar stage[a]	n	Slope ± SE	LT_{50} (95% CL)	$LT_{99.9968}$ (95% CL)[b]
Mediterranean fruit fly	Feeding	1,900	18.2 ± 2.1	10.9 (9.5–12.5)a	15.5 (13.1–28.1)a
	Nonfeeding	2,050	11.9 ± 1.3	5.9 (4.9–8.2)b	8.4 (6.6–19.1)a
Melon fly	Feeding	2,000	15.2 ± 1.1	11.4 (10.8–12.0)a	15.5 (14.3–17.3)a
	Nonfeeding	2,000	51.9 ([c])	11.2 (10.6–11.9)a	15.3 (14.1–17.3)a
Oriental fruit fly	Feeding	2,000	22.6 ± 7.6	12.9 (12.2–13.5)a	16.9 (16.1–18.2)a
	Nonfeeding	2,000	17.8 ± 2.1	10.7 (10.3–11.2)b	14.0 (13.0–15.8)b

For each species, significant differences between feeding and nonfeeding stages were determined by nonoverlap of 95% CL.
[a] Feeding larvae were removed directly from rearing diet; nonfeeding larvae were collected after they left the rearing diet before beginning pupation.
[b] Probit 9 response.
[c] SE of slope not calculable because of poor fit.

Discussion

Litchi fruit is a relatively poor host for Mediterranean fruit fly and oriental fruit fly. Litchi is not recorded as a host for melon fly. Table 1 indicates that immersing litchi in 49°C water for 20 min followed by hydrocooling in ambient (24 ± 4°C) temperature water for 20 min provides probit nine quarantine security against Mediterranean fruit fly, melon fly, and oriental fruit fly eggs and larvae. Table 4 shows that the temperature profile for the 20-min 49°C hot-water immersion treatment followed by 20-min hydrocooling in ambient temperature water killed 100% of 15,000 feeding and 15,000 nonfeeding third instars for each of the three fruit fly species. Eggs and first instars are more heat tolerant than the third instars for the three fruit fly species (Armstrong et al. 1989). Therefore, the treatment efficacy shown in Tables 1 and 4 support the use of the 49°C hot-water immersion for 20 min, followed by hydrocooling in ambient temperature water for 20 min, as a quarantine treatment for Hawaii-grown litchi fruit before export to the U.S. mainland to provide quarantine security against any potential infestations of Mediterranean fruit fly or oriental fruit fly eggs or larvae. The research reported here is the basis for the USDA–APHIS approved hot-water immersion quarantine treatment for litchi (Federal Register 1997).

Preliminary information suggested the hot-water immersion treatment would not harm litchi quality. In general, litchis have a short storage life under ambient conditions. Dessication with accompanying loss of red color and development of browning can occur rapidly (<72 h) (Nip 1988). Lowering the storage temperature is proven to extend the shelf life. However, research with Kaimana litchi in Hawaii demonstrated that the hot-water immersion treatment accelerated fruit darkening during cold storage at 4°C (Follett and Sanxter 2003), which could affect its marketability. Various other techniques have been tested to prolong shelf life, including packaging, fumigation, chemical treatments, and modified atmospheres with reportedly impressive results (Nip 1988). Further research is needed to identify treatments and procedures to slow the rate of quality loss in litchi after hot-water immersion treatment.

The hot-water immersion treatment also causes some darkening in longan fruit, but this is less of a problem for marketability because the fruit is naturally brown. Hot-water immersion should be an acceptable treatment for longan when coupled with cold storage at 2–5°C (Follett and Sanxter 2002). Longan fruit is typically smaller than litchi fruit. In a 2-yr survey of Hawaii orchards, the average weight of litchi fruit was 17.2 g (max = 39 g; n = 32,000) and longan fruit averaged 9.4 g (max = 22 g; n = 9,700) (G. McQuate, USDA–ARS, unpublished data). USDA–APHIS approved a hot-water immersion treatment for longan exported from Hawaii to the U.S. mainland based on the research reported here for litchi (Federal Register 2002), and the fact that smaller longan fruit heat faster than litchi fruit (Fig. 1).

Cryptophlebia illepida (Butler) and C. *ombrodelta* (Lower) are internal-feeding moth pests that infest litchi and longan. Current regulations for litchi and longan hot-water immersion treatment stipulate that fruit must be found free of *Cryptophlebia.* Research showed that the hot-water immersion treatment will effectively disinfest litchi and longan of any *Cryptophlebia* in addition to fruit flies (Follett and Sanxter 2001).

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